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New cytotoxic flavonoids from *Cryptocarya infectoria*

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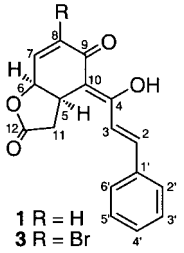
Abstract—A new dihydrochalcone, two new dihydroflavanones and eight new biflavonoids have been isolated together with cryptocaryone from the cytotoxic methanol extract of *Cryptocarya infectoria*. The absolute structure of cryptocaryone was established by X-ray analysis of its 8-bromo derivative. The structure of the new compounds were elucidated by spectroscopic means and their absolute stereochemistry was deduced from chemical correlation, circular dichroism data and NOESY experiments. Several compounds displayed significant cytotoxicity and cryptocaryone was shown to possess cytotoxicity towards multi-drug resistant K562-DOX cells.

1. Introduction

The genus *Cryptocarya* belongs to the pantropical family Lauraceae and most of the species grow in the Pacific–Asian tropical rainforests. Many of these species which have been examined for their chemical constituents contain flavonoids, alkaloids and 6-alkyl- and 6-aryl-α-pyrones.[1] In the course of our continuing search for plant natural compounds that have biological activities, a crude extract of the trunk bark of *Cryptocarya infectoria* (Bl.) Miq. collected in the south of Hanoi (North Vietnam)[†] was found cytotoxic against KB cells. To our knowledge, this species had never been studied. In this paper, we report the isolation and cytotoxicity of cryptocaryone **1**, one new dihydrochalcone **4**, two new dihydroflavanones **5** and **6** and eight new biflavonoids[‡] **8**–**15**.



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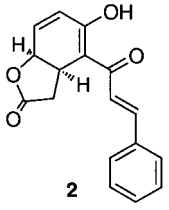
3 R = Br



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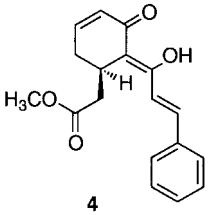

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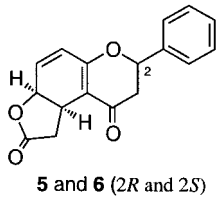
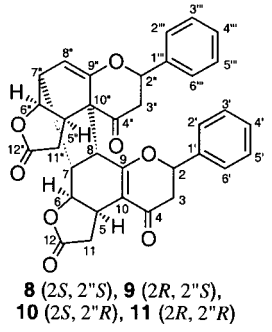
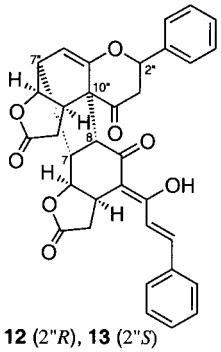


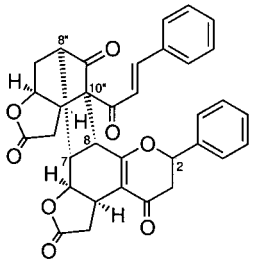
5 and **6** (2*R* and 2*S*)



8 (2*S*, 2"*S*), **9** (2*R*, 2"*S*),
10 (2*S*, 2"*R*), **11** (2*R*, 2"*R*)



12 (2"*R*), **13** (2"*S*)



14 (2*S*), **15** (2*R*)

Keywords: *Cryptocarya infectoria*; biflavonoids; cytotoxicity.

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† Plant collected by one of us (V. D.) in the framework of the collaborative program between CNRS (France) and NCST (Vietnam).

‡ Taking into account that the isolated biflavonoids come from the condensation of cryptocaryone **1** with its flavanone form, the numbering of all compounds is given from the system used for flavanone and not from that of chalcone type.

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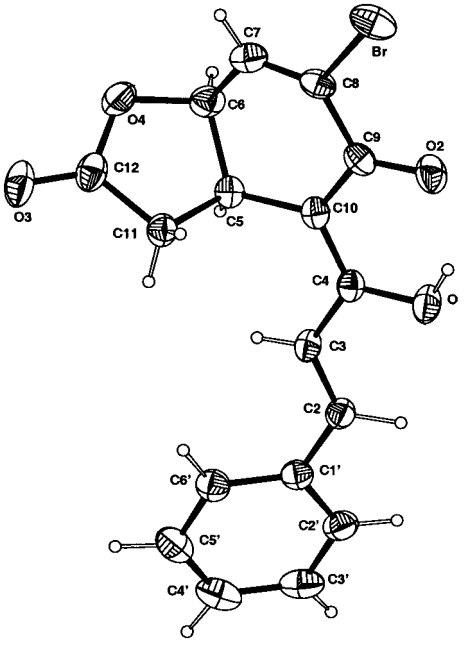


Figure 1. ORTEP drawing of bromocryptocaryone **3**.

2. Results and discussion

The known cryptocaryone **1** was obtained as the main compound of the crude extract. This dihydrochalcone was initially isolated in 1972 from another species of *Cryptocarya* (*C. bourdilloni*),[2] and its structure was first formulated as **2** from spectral data and chemical degradation and correlation.[3] Later on, its structure was revised as the keto–enol tautomeric form **1** (relative configuration) after X-ray crystallographic analysis.[4] In order to assign the absolute configuration of cryptocaryone **1**, we prepared the 8-bromocryptocaryone **3** derivative obtained after treatment of cryptocaryone **1** by bromine. X-ray analysis of this compound (Fig. 1) gave the absolute configuration 5*R*,6*S* of cryptocaryone **1**.

The primary structure of compounds **4** (infectocaryone), **5** and **6** (cryptocaryanones A and B) was deduced from the IR, mass and 1D (Tables 1 and 2) and 2D NMR spectra by comparison with those of cryptocaryone **1**.

Infectocaryone **4** showed a molecular ion at *m/z* 298 and a similar fragmentation pattern to that of cryptocaryone **1**. A comparison of the ^{13}C and ^{1}H NMR spectra of **4** with that of cryptocaryone **1** revealed the structural similarity of these two compounds, the only difference being the appearance in **4** of a methoxy and methylene group at C-12 and C-6, respectively, in place of the γ-lactone group (Tables 1 and 2). The absence of the γ-lactone group was confirmed from the IR spectrum (no band at 1780 cm^{-1}). The keto–enol tautomeric structure of infectocaryone **4** was assigned by comparison of its HMBC correlations with those of cryptocaryone. The CD data of compound **4** are similar to those of cryptocaryone **1**, fixing the absolute stereochemistry at C-5.

The flavanone skeleton of cryptocaryanones A **5** and B **6** was deduced from their ^{1}H NMR spectra which revealed the presence of an ABX system at δ 5.48, 2.93, 2.72 and δ 5.43, 2.92, 2.72, respectively, instead of a set of *trans*-olefinic protons found in **1** and **4** (Table 1). The configuration at carbons 5 and 6 in cryptocaryanone A **5** and cryptocaryanone B **6** was determined by chemical correlation with cryptocaryone **1**. Thus, the reaction of cryptocaryone **1** with acetic acid in aqueous ethanol under reflux gave a mixture of flavanones **5**, **6** and racemic **7** (Scheme 1). The configuration at carbon 2 in the two epimers **5** and **6** was assigned as 2*R* for **5** and 2*S* for **6** by comparison of their circular dichroism spectra with those of flavanones.[5] Thus, the observed negative Cotton effect of the $n\rightarrow\pi^*$ transition in the 330–370 nm region of compound **5** reflects a 2*R*-configuration whereas the positive Cotton effect of compound **6** is reminiscent of a 2*S*-configuration. It should be noted that compounds **5** and **6** represent the first examples of natural flavanones bearing a reduced A ring.

The molecular formula, $C_{34}H_{28}O_8$, for bicaryanones A **8**, B **9**, C **10** and D **11**, was derived from their EIMS (*m/z* 564 $[M]^+$) and their HRCIMS. They each possess four characteristic IR bands corresponding to two γ-lactones, one conjugated ketone and one ketone. The ^{1}H NMR and ^{13}C NMR spectra of the four biflavonoids **8**–**11** (Tables 3 and 4) were all very similar and showed them to be related to cryptocaryanones A **5** and B **6**. The most notable differences were the presence of signals attributable to two aromatic signals and only one olefinic hydrogen in the ^{1}H NMR

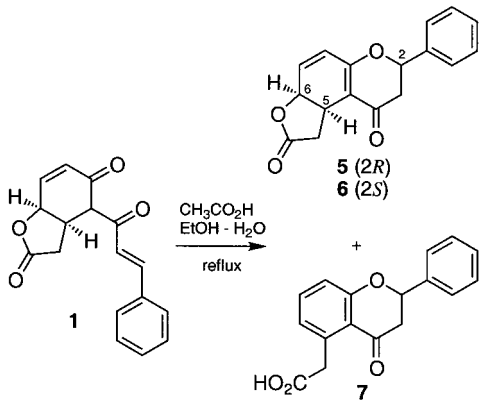
Table 1. ^{1}H NMR data for **1**, **4**, **5** and **6**

Proton	Cryptocaryone **1**	Infectocaryone **4**	Cryptocaryanone A **5**	Cryptocaryanone B **6**
H-2	7.75 d (14)	7.69 d (15)	5.48 dd (13, 3)	5.44 dd (14, 4)
H-3α	6.80 d (14)	7.02 d (15)	2.72 dd (16, 3)	2.92 dd (17, 14)
H-3β			2.93 dd (16, 13)	2.72 dd (17, 4)
H-5	3.99 ddd (11, 8, 8)	3.60 m	3.87 dt (10, 9)	3.78 dt (9, 8)
H-6α	5.46 dd (8, 2)	2.43 dd (18, 7)	5.48 ddd (9, 3, 1)	5.43 ddd (9, 4, 1)
H-6β		2.65 ddd (18, 6, 3)		
H-7	6.54 bd (9)	6.70 ddd (10, 7, 3)	6.28 dd (9, 3)	6.37 dd (10, 4)
H-8	6.18 dd (9, 2)	6.18 dd (10, 3)	6.09 dd (11, 1)	6.11 dd (10, 1)
H-11α	2.59 dd (16, 11)	2.41 dd (15, 10)	2.39 dd (17, 10)	2.65 dd (18, 8)
H-11β	2.78 dd (16, 8)	2.62 dd (15, 5)	2.98 dd (17, 9)	3.04 dd (18, 9)
H-2′	7.56 m	7.57 m	7.41 m	7.43 m
H-3′	7.40 m	7.39 m	7.41 m	7.43 m
H-4′	7.40 m	7.39 m	7.41 m	7.43 m
H-5′	7.40 m	7.39	7.41 m	7.43 m
H-6′	7.56 m	7.57 m	7.41 m	7.43 m
OH-4	17.00 s	13.66 s		
CH_3O-12		3.65 s		

Table 2. ^{13}C NMR data for **1**, **4**, **5** and **6**

Carbon	Cryptocaryone **1**	Infectocaryone **4**	Cryptocaryanone A **5**	Cryptocaryanone B **6**
C-2	142.2	140.4	80.8	80.6
C-3	116.9	118.1	42.7	42.4
C-4	174.1	172.6	190.2	190.2
C-5	33.7	29.7	30.5	30.7
C-6	76.2	29.6	76.7	77.1
C-7	140.2	144.1	134.6	133.9
C-8	130.0	129.4	124.1	125.2
C-9	185.8	188.3	162.5	162.8
C-10	103.5	108.4	108.8	109.3
C-11	35.2	40.0	33.1	34.5
C-12	174.6	172.7	175.4	175.4
C-13		51.9		
C-1′	134.8	135.5	137.5	137.5
C-2′	128.3	128.1	126.3	126.3
C-3′	129.0	129.0	128.9	129.1
C-4′	130.5	130.0	128.9	129.1
C-5′	129.0	129.0	128.9	129.1
C-6′	128.3	128.1	126.3	126.3

spectrum of **8**–**11** instead of one and two, respectively, as in **5** and **6**. 2D NMR experiments (COSY and HMBC) allowed to establish the presence of two interflavonyl bonds and their mixed positions at C-7–C-7″ and C-8–C-10″ as shown in structures **8**, **9**, **10** and **11**. A plausible biogenesis of these biflavonoids would be a Diels–Alder cycloaddition type reaction occurring between two identical cryptocaryanone molecules (**5** or **6**) or between **5** and **6** leading thus to four diastereoisomers **8**–**11**. The orientation of H-5, H-6 and H-5″, H-6″ can thus be assigned as α. This was confirmed for bicaryanone D **11** by its X-ray crystallographic analysis as shown in Fig. 2. The X-ray structure of **11** led also to the determination of the configuration of the six other stereocentres at C-2, C-7, C-8, C-2″, C-7″ and C-10″. The configuration at C-7, C-8, C-7″ and C-10″ in compounds **8**, **9** and **10** was deduced from their NOESY spectra. Thus, the presence of nOe correlations between H-8/5″, H-5/8″ and H-7/6″ indicated a β orientation of the hydrogens at C-7, C-8 and C-7″. The configuration at carbons 2 and 2″ of the three biflavonoids **8**, **9** and **10** was determined from their NOESY NMR spectrum by comparison with that of compound **11**. The presence of a nOe cross-peak between H-2″/11″β in bicaryanones **8** and **9** fixed the β position of their H-2″ whereas a nOe association between H-2″/3α in **10** supported the α positional assignment of H-2″ in these two compounds. In addition nOe cross-peaks between H-2/3″α and H-2/2‴ in **9** and H-2″/H-3α in **10** permitted to establish the configuration at C-2. According to these data and the X-ray analysis of **11** (2*R*, 2″*R*), compounds **8**, **9** and **10** have, respectively, a (2*S*,2″*S*), (2*R*,2″*S*) and (2*S*,2″*R*) configuration.



Scheme 1.

Chalcocaryanones A **12** and B **13** exhibited in their mass spectra a molecular ion at *m*/*z* 564 as in compounds **8**–**11**. An exchangeable singlet observed at δ 16.40 (compound **12**) and 16.57 ppm (compound **13**) in their ^{1}H NMR spectra (Table 5) and the ^{13}C NMR resonances (Table 6) observed at δ 179.1 (C-4), 104.1 (C-10), 193.1 (C-9) for compound **12** and δ 178.9 (C-4), 105.1 (C-10), 190.4 (C-9) for compound **13** were indicative of the presence of a β-diketone trapped in the enol form. In addition, a set of *trans*-olefinic protons (positions 2 and 3) and an ABX system (position 2″ and 3″) was observed in the ^{1}H NMR spectra of **12** and **13** (Table 5). These data are in agreement with dimeric structures formed from cryptocaryone **1** and cryptocaryanones **5** and **6**. The interflavonyl linkages (C-7–C-7″ and C-8–C-10″) were determined by heteronuclear multiple bond connectivities (HMBC) and comparison with those of biflavonoids **8**, **9**, **10** and **11**. The β position of H-2″ in **13** was established by the presence of a nOe cross-peak between H-2″ and H-11″β.

Chalcocaryanones C **14** and D **15** were found to have a molecular ion at *m*/*z* 564. Analysis of the ^{1}H NMR spectrum of the two compounds indicated the presence of a flavanone and a chalcone moiety characterized, respectively, by the presence of ABX signals at δ 5.20, 2.55, 2.81 ppm in **14** and δ 5.08, 2.54, 2.85 ppm in **15** and two *trans* cinnamoyl hydrogens at δ 7.77 and 7.27 ppm in **14** and at δ 7.11 and 6.98 ppm in **15** (Table 5). The interflavonyl linkage between the two building blocks was different from that found in compounds **8**–**13**. In contrast to these compounds, the ^{1}H NMR spectra of chalcocaryanones A **14** and B **15** lacks the doublet corresponding to the olefinic hydrogen at carbon 8″ which was replaced by a methine hydrogen. In addition, the spectra of **14** and **15** showed signals of methylene hydrogens at carbon 7″ instead of a methine hydrogen in **8**–**13**. The HMBC spectrum of **14** and **15** shows some important correlations. For example, the hydrogen H-8 exhibited correlation with C-4″, C-5″, C-8″, C-9″ and C-10″ whereas

Table 3. ^{1}H NMR data for bicaryanones A (**8**), B (**9**), C (**10**) and D (**11**)

Proton	8	9	10	11
H-2	5.36 dd (14.3, 3.7)	5.45 dd (11.7, 4.2)	5.49 dd (14.4, 4.3)	5.46 dd (13.5, 3.4)
H-3α	2.92 dd (17.2, 14.3)	2.70 dd (16.8, 4.2)	2.92 dd (17.6, 14.4)	2.70 dd (17.2, 3.4)
H-3β	2.84 dd (17.2, 3.7)	2.83 dd (16.8, 11.7)	3.00 dd (17.6, 4.3)	2.85 dd (17.2, 13.5)
H-5	3.27 brdd (6.9, 4.7)	3.37 ddd (7.7, 5.2, 4.5)	3.33 m	3.67 ddd (11.7, 8.1, 7.5)
H-6	4.68 dd (4.7, 2.2)	4.57 dd (5.2, 4.4)	4.63 dd (5.0, 2.8)	4.42 t (7.5)
H-7	2.73 brd (8.6)	2.55 brdd (8.9, 4.4)	2.63 brdd (9.9, 2.8)	2.37 brdd (10.5, 7.5)
H-8	3.34 d (8.6)	3.49 d (8.9)	3.39 d (9.9)	3.57 d (10.5)
H-11α	2.95 dd (17.7, 6.9)	2.85 dd (17.8, 7.7)	2.94 dd (17.6, 7.2)	2.95 dd (17.4, 8,1)
H-11β	2.83 dd (17.7, 1.3)	2.51 dd (17.8, 4.5)	2.76 dd (17.6, 2.6)	2.15 dd (17.4, 11.7)
H-2′	7.22 m	7.11 m	7.34 m	7.32 m
H-3′	7.33 m	7.31 m	7.45 m	7.44 m
H-4′	7.33 m	7.31 m	7.45 m	7.44 m
H-5′	7.33 m	7.31 m	7.45 m	7.44 m
H-6′	7.22 m	7.11 m	7.34 m	7.32 m
H-2″	4.90 dd (10.8, 4.7)	4.94 dd (11.7, 4.2)	4.93 dd (10.8, 3.8)	4.80 dd (11.5, 2.8)
H-3″α	2.57 dd (18.0, 10.8)	2.63 dd (18.4, 11.7)	2.39 dd (18.4, 3.8)	2.37 dd (18.4, 2.8)
H-3″β	2.51 dd (18.0, 4.7)	2.55 dd (18.4, 4.2)	2.32 dd (18.4, 10.8)	2.28 dd (18.4, 11.5)
H-5″	3.17 ddd (10.8, 7.6, 2.7)	3.12 ddd (10.6, 7.9, 2.7)	3.21 ddd (10.9, 7.7, 4.1)	3.22 ddd (10.9, 8.1, 4.9)
H-6″	4.93 dd (7.6, 3.9)	4.91 dd (7.9, 4.1)	4.88 dd (7.7, 3.6)	4.85 dd (8.1, 3.0)
H-7″	3.19 brdd (6.9, 3.9)	3.27 dd (6.8, 4.1)	3.19 brdd (6.8, 3.6)	3.44 m
H-8″	5.30 d (6.9)	5.38 d (6.8)	5.26 d (6.8)	5.43 d (6.9)
H-11″α	2.82 dd (19.0, 10.8)	2.80 dd (19.0, 10.6)	2.61 dd (18.9, 10.9)	2.59 dd (18.8, 10.9)
H-11″β	2.06 dd (19.0, 2.7)	2.09 dd (19.0, 2.7)	2.07 dd (18.9, 4.1)	2.10 dd (18.8, 4.9)
H-2‴	7.16 m	7.20 m	7.03 m	7.00 m
H-3‴	7.37 m	7.37 m	7.34 m	7.32 m
H-4‴	7.37 m	7.37 m	7.34 m	7.32 m
H-5‴	7.37 m	7.37 m	7.34 m	7.32 m
H-6‴	7.16 m	7.20 m	7.03 m	7.00 m

Table 4. ^{13}C NMR data for bicaryanones A (**8**), B (**9**), C (**10**) and D (**11**)

Carbon	8	9	10	11
C-2	79.3	79.3	79.8	78.1
C-3	40.5	43.6	42.6	43.6
C-4	190.7	190.7	190.0	190.2
C-5	33.0	32.4	32.6	32.4
C-6	80.0	80.5	81.5	80.8
C-7	36.7	37.6	37.2	39.4
C-8	45.1	46.3	43.0	45.6
C-9	168.4	166.5	168.6	166.3
C-10	112.4	112.3	112.9	112.7
C-11	37.7	35.6	36.3	33.8
C-12	175.2	175.1	175.0	176.0
C-1′	135.6	136.5	135.6	136.2
C-2′	126.1	125.6	126.1	125.6
C-3′	129.0	129.1	129.1	129.3
C-4′	129.4	129.3	129.7	129.7
C-5′	129.0	129.1	129.1	129.3
C-6′	126.1	125.6	126.1	125.6
C-2″	75.9	76.1	75.9	76.1
C-3″	47.1	45.9	48.8	48.6
C-4″	204.7	204.6	203.5	203.9
C-5″	40.5	41.1	40.6	41.1
C-6″	80.5	81.0	81.5	82.2
C-7″	38.7	38.8	39.1	39.5
C-8″	100.6	101.5	99.2	100.3
C-9″	153.6	154.1	153.8	154.4
C-10″	53.4	52.6	52.9	51.3
C-11″	32.3	32.2	30.5	30.4
C-12″	174.7	174.6	174.5	175.2
C-1‴	138.1	138.0	138.1	138.0
C-2‴	125.5	125.6	125.2	125.1
C-3‴	128.9	128.9	128.9	128.8
C-4‴	128.9	128.9	128.9	128.8
C-5‴	128.9	128.9	128.9	128.8
C-6‴	125.5	125.6	125.2	125.1

H-7 shows a correlation with C-7″ and C-11″. All these data suggest a mixed interflavonyl linkage between C-7–C-8″ and C-8–C-10″. Finally, the position of H-2 was fixed as β for compound **14** and α for **15** from NOESY experiments which gave a nOe effect between H-2, H-11β and H-3 in compound **15**.

Compounds **1** and **4**–**15** were evaluated against KB cell lines. The monomers **1**, **4**, **5** and **6** were cytotoxic, yielding IC_{50} values of 1.8, 1.7, 2.5 and 2.1 μM, respectively. In contrast, the eight biflavonoids **8**–**15** as well as compound **7** were inactive. Cryptocaryone **1** also exhibit cytotoxicity against erythroleukemic K562 and doxorubicin-resistant K562 cells with the same IC_{50} value of 2 μM. 5,6-dihydrochalcones and 5,6-flavanones such as **1**, **4**, **5** and **6** can thus be considered as new series of potential antitumor agents.

3. Experimental

Optical rotations at 20°C were measured on a Perkin–Elmer 241 polarimeter. UV spectra were recorded on a Varian Cary 100 spectrometer and IR on a Perkin–Elmer Spectrum BX FT-IR spectrometer; HRCI and EI mass spectra were recorded on a Kratos MS 80 or MS 50, respectively. CD spectra were recorded on a Jobin Yvon CD6 dichrograph. The NMR spectra were recorded in $CDCl_3$ on Bruker AC 250, AC 300 or AMX 400 spectrometers. Chemical shifts (relative to TMS) are in ppm and coupling constants (in parentheses) in Hz. Column chromatography (CC) was performed using silica gel Merck H60. Preparative plates (PLC) [silica gel 60 F_{254}] were also used for purification. Preparative HPLC was performed on a Waters PrepPak cartridge (Porasil® 15–20 μm 125 Å, 57×300 mm) at 50 mL/min using a Waters Delta prep 3000 apparatus. Semi-

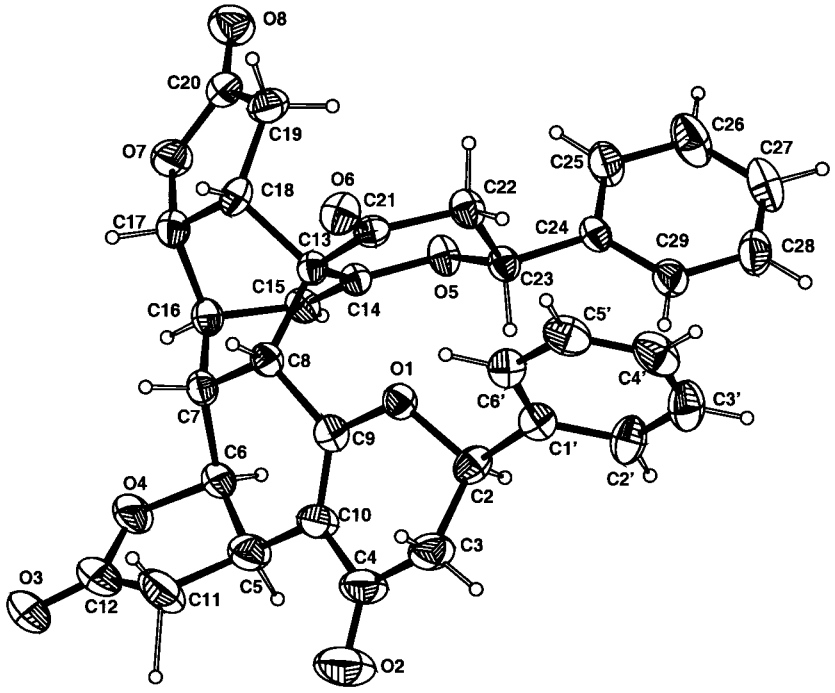


Figure 2. ORTEP drawing of bicaryanone D **11**.

preparative HPLC was carried out on Waters RCM (Prep Nova-Pak® HR silica 6 μm 60 Å, 25×100 mm) at 10 mL/min.

3.1. Plant material

Trunk bark of *Cryptocarya infectoria* (Bl.) Miq. was collected at Song Chang, Nhu Xuan, Thanh Hoa province, 150 km south of Hanoi, North Vietnam, in April 1996. Identification was provided by one of us (V. D.) and Tran Ngoc NINH, Institute of Ecology and Biological Resources, N.C.S.T., Hanoi, Vietnam. Voucher specimens (VN 105) are deposited in the Herbarium of that Institute.

3.2. Extraction and isolation

The dried ground trunk bark of *Cryptocarya infectoria*

Table 5. ^{1}H NMR data for chalcocaryanones A (**12**), B (**13**), C (**14**) and D (**15**)

Proton	**12**	**13**	Proton	**14**	**15**
H-2	7.87 d (15.5)	7.86 d (15.4)	H-2	5.20 dd (15.3, 2.8)	5.08 dd (13.8, 3.4)
H-3	6.72 d (15.5)	6.71 d (15.4)	H-3α	2.81 dd (17.3, 15.3)	2.85 dd (16.5, 13.8)
H-5	3.68 ddd (11.3, 7.5, 7.0)	3.63 brq (7.0)	H-3β	2.55 dd (17.3, 2.8)	2.54 dd (16.5, 3.4)
H-6	4.53 t (7.0)	4.58 t (5.7)	H-5	3.42 brt (6.2)	3.49 brt (6.1)
H-7	2.44 brdd (11.1, 7.0)	2.58 brdd (9.9, 5.7)	H-6	4.80 dd (4.8, 2.3)	4.77 dd (4.8, 2.3)
H-8	3.59 d (11.1)	3.46 d (9.9)	H-7	2.87 brd (8.7)	2.87 brd (9.5)
H-11α	2.92 dd (17.2, 7.5)	2.94 dd (17.0, 7.2)	H-8	4.06 d (8.7)	4.12 d (9.5)
H-11β	2.49 dd (17.2, 11.3)	2.48 dd (17.0, 7.5)	H-11α	3.06 dd (17.8, 7.4)	2.95 dd (18.0, 7.5)
H-2′	7.58 m	7.57 m	H-11β	2.79 brd (17.8)	2.73 brd (18.0)
H-3′	7.46 m	7.42 m	H-2′	7.16 m	7.11 m
H-4′	7.46 m	7.42 m	H-3′	6.88 m	6.98 m
H-5′	7.46 m	7.42 m	H-4′	6.88 m	6.90 m
H-6′	7.58 m	7.57 m	H-5′	6.88 m	6.98 m
H-2″	5.22 dd (11.6, 2.3)	4.99 dd (12.6, 2.5)	H-6′	7.16 m	7.11 m
H-3″α	3.21 dd (18.1, 2.3)	3.35 dd (17.5, 12.6)	H-2″	7.77 d (15.7)	7.64 d (15.7)
H-3″β	2.56 dd (18.1, 11.6)	2.79 dd (17.5, 2.5)	H-3″	7.27 d (15.7)	7.24 d (15.7)
H-5″	3.21 ddd (10.8, 7.9, 4.7)	3.23 ddd (11.1, 8.1, 3.4)	H-5″	3.36 ddd (11.1, 8.4, 2.9)	3.38 ddd (11.0, 8.6, 2.9)
H-6″	4.86 dd (7.9, 3.1)	4.89 dd (8.1, 3.5)	H-6″	4.92 brdd (8.4, 4.0)	4.91 brdd (8.3, 4.0)
H-7″	3.40 ddd (6.8, 3.1, 1.8)	3.37 m	H-7″α	2.67 brd (20.5)	2.66 ddd (20.4, 9.4, 3.6)
H-8″	5.35 d (6.8)	5.38 d (6.8)	H-7″β	2.22 brdd (20.5, 3.2)	2.27 brd (19.0)
H-11″α	2.66 dd (18.8, 10.8)	2.83 dd (19.0, 11.1)	H-8″	2.87 m	2.87 m
H-11″β	2.22 dd (18.8, 4.7)	2.12 dd (19.0, 3.4)	H-11″α	2.70 dd (19.3, 11.1)	2.69 dd (19.3, 11.0)
H-2‴	7.38 m	7.34 m	H-11″β	2.38 dd (19.3, 2.9)	2.31 dd (19.3, 2.9)
H-3‴	7.38 m	7.34 m	H-2‴	7.44 m	7.27 m
H-4‴	7.38 m	7.34 m	H-3‴	7.44 m	7.31 m
H-5‴	7.38 m	7.34 m	H-4‴	7.44 m	7.36 m
H-6‴	7.38 m	7.34 m	H-5‴	7.44 m	7.31 m
OH-4	16.40 s	16.57 s	H-5‴	7.44 m	7.27 m

Table 6. ^{13}C NMR data for chalcocaryanones A (**12**), B (**13**), C (**14**) and D (**15**)

Carbon	12	13	14	15
C-2	145.4	144.5	80.9	82.8
C-3	116.9	117.6	41.8	43.8
C-4	179.1	178.9	190.8	191.4
C-5	35.5	35.2	32.8	32.7
C-6	78.7	79.2	79.3	79.4
C-7	38.6	38.5	33.7	33.9
C-8	51.8	52.7	38.4	39.5
C-9	193.1	190.4	169.0	168.2
C-10	104.1	105.1	111.0	110.7
C-11	36.2	37.8	37.7	36.5
C-12	174.8	174.9	174.6	174.7
C-1′	134.4	134.4	135.5	136.4
C-2′	128.7	128.6	127.0	125.9
C-3′	129.3	129.2	128.4	128.6
C-4′	131.5	131.2	128.8	129.0
C-5′	129.3	129.2	128.4	128.6
C-6′	128.7	128.6	127.0	125.9
C-2″	76.9	76.7	145.1	145.0
C-3″	49.1	47.5	124.0	123.7
C-4″	204.2	205.5	195.1	195.6
C-5″	43.0	41.0	41.7	41.2
C-6″	81.2	80.5	78.4	78.4
C-7″	39.5	39.1	36.3	35.9
C-8″	99.0	100.2	34.7	34.9
C-9″	155.8	154.7	205.9	206.7
C-10″	51.2	52.4	62.9	62.8
C-11″	30.4	32.3	30.1	30.1
C-12″	174.9	175.4	173.9	174.0
C-1‴	138.8	138.5	134.5	134.4
C-2‴	125.9	126.0	128.8	128.9
C-3‴	129.0	128.9	129.1	129.2
C-4‴	128.8	128.6	131.1	130.9
C-5‴	129.0	128.9	129.1	129.2
C-6‴	125.9	126.0	128.8	128.9

(3.1 kg) was extracted at room temperature with methanol and the solvent was evaporated under vacuum to give a crude extract (200 g) which exhibits 98% inhibition on KB cells at 10 μg/mL. An aliquot (26 g) of this residue was subjected to CC (silica gel) using a step gradient of AcOEt/heptane (3:7)–MeOH to give 16 fractions. Fraction 5 (0.449 g) was again separated on CC (silica gel) using heptane/acetone (8:2) as eluent followed by preparative TLC on silica plates developed with AcOEt to yielded infectocaryone **4** (0.150 g). Fraction 7 (1.44 g) was purified on CC (silica gel) using heptane/acetone (7:3) and afforded bright yellow crystals of cryptocaryone **1** (1.11 g). Fraction 10 (0.562 mg) was worked up as fraction 7 with CH_2Cl_2/MeOH (0.5%) and final purification was achieved by semipreparative HPLC with CH_2Cl_2 as eluent to yield cryptocaryanone A **5** (5.5 mg) and cryptocaryanone B **6** (10.8 mg). A second part of the crude extract (62.8 g) was extracted with CH_2Cl_2. The soluble fraction (12.4 g) was subjected to CC (silica gel) with CH_2Cl_2/MeOH (98:2) and one of the obtained fraction was extracted with heptane/acetone (9:1). The insoluble residue (3.49 g) was run through preparative HPLC with heptane–AcOEt–HOAc (60:40:0.3) and further separated by preparative or semi-preparative HPLC to afford bicaryanone A **8** (122 mg, heptane–AcOEt–HOAc 60:40:0.3), bicaryanones B **9** and C **10** (26 and 51 mg, respectively, heptane–AcOEt–HOAc 50:50:0.3), bicaryanone D **11**, chalcocaryanones A **12**, B **13**, C **14** and D **15** (54, 10, 37, 10 and 68 mg, respectively, heptane–AcOEt–HOAc 60:40:0.1).

3.2.1. Cryptocaryone 1. Yellow needles from diethyl ether, mp 153°C (lit.[2] 153°C); $[\alpha]_D^{25}=+770.7°$ (*c* 0.99, $CHCl_3$) [lit. +776.6° (*c* 2, $CHCl_3$)[2]]; UV (EtOH) λ_{max} (ϵ) 397 (22841), 385 (22973), 288 (11057), 236 (10958), 202 (19060) nm; IR ($CHCl_3$) ν_{max} 3565, 1783, 1630, 1580, 1416, 1321, 1171, 1024, 895 cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 75 MHz) see Table 2; EIMS *m/z* 282 $[M]^{+\cdot}$ (100), 223 (58), 131 (81), 104 (58), 103 (69), 91 (20), 77 (65); CD (EtOH) λ_{ext} ($\Delta\epsilon$) 204 (+9.2), 222 (−3.6), 239 (−2.2), 254 (+2.3), 293 (−1.9), 389 (+8.7).

3.2.2. Bromocryptocaryone 3. A sample of cryptocaryone (100 mg) was stirred in THF (3 mL), treated with $KHCO_3$ (36 mg) and 18 μL of Br_2 was added at 0°C. After removal of the solvent in vacuo, the residue was dissolved in CH_2Cl_2 and washed with water. The organic mixture was fractionated over CC (silica gel) eluted with CH_2Cl_2 and submitted to repeated preparative TLC with CH_2Cl_2/MeOH (0.3%) to give 8-bromocryptocaryone (42 mg). Recrystallization from AcOEt gave unstable orange needles (Fig. 1, Table 7). $[\alpha]_D^{25}=+462.2°$ (*c* 0.99, $CHCl_3$); UV (EtOH) λ_{max} (ϵ) 406 (20164), 295 (16107), 201 (30111) nm; IR ($CHCl_3$) ν_{max} 3567, 1790, 1628, 1580, 1553, 1402, 1333, 1169, 1005, 890 cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) δ 2.62 (1H, dd, *J*=18, 12 Hz, H-11α), 2.81 (1H, dd, *J*=16, 8 Hz, H-11β), 4.01 (1H, bq, *J*=10 Hz, H-5), 5.42 (1H, d, *J*=7 Hz, H-6α), 7.01 (1H, bs, H-7), 6.79 (1H, d, *J*=16 Hz, H-3), 7.41 (3H, m, H-3′, H-4′, H-5′), 7.58 (2H, m, H-2′, H-6′), 7.80 (1H, d, *J*=14 Hz, H-2), 13.63 (1H, s, OH-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ 34.4 (C-5), 35.3 (C-11), 76.7 (C-6), 102.6 (C-10), 116.1 (C-3), 126.0 (C-8), 128.5 (C-2′ and C-6′), 129.3 (C-3′ and C-5′), 131.0 (C-4′), 134.7 (C-1′), 140.8 (C-7), 143.5 (C-2), 173.3 (C-4), 174.0 (C-12), 181.0 (C-9); EIMS *m/z* 360 $[M]^{+\cdot}$ (5), 362 (5), 240 (26), 238 (28), 230 (29), 228 (30), 227 (21), 225 (19), 131 (72), 104 (66), 103 (83), 91 (24), 77 (100); HREIMS *m/z* 360.0001 (calcd for $C_{17}H_{13}O_4Br$, 359.9997).

3.2.3. Infectocaryone 4. Amorphous yellow powder, $[\alpha]_D^{25}=+128.0°$ (*c* 0.94, $CHCl_3$); UV (EtOH) λ_{max} (ϵ) 383 (22106), 281 (10748), 242 (8624), 235 (9559), 200 (21174) nm; IR ($CHCl_3$) ν_{max} 3564, 1732, 1672, 1631, 1652, 1603, 1579, 1438, 1282, 1168, 972 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 75 MHz) see Table 2; EIMS *m/z* 298 $[M]^{+\cdot}$ (22), 225 (48), 131 (100), 121 (49), 103 (63), 91 (30), 77 (56); HRCIMS *m/z* 299.1294 (calcd for $C_{18}H_{19}O_4$, 299.1283). CD (EtOH) λ_{ext} ($\Delta\epsilon$) 225 (−13.3), 275 (+2.0), 320 (−0.6), 374 (+3.3).

3.2.4. Cryptocaryanone A 5. Amorphous yellow powder, $[\alpha]_D^{25}=+184.0°$ (*c* 0.97, $CHCl_3$); UV (EtOH) λ_{max} (ϵ) 323 (7456), 201 (19038) nm; IR ($CHCl_3$) ν_{max} 1784, 1661, 1591, 1428, 1326, 1278, 1019, 896 cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 75 MHz) see Table 2; EIMS *m/z* 282 $[M]^{+\cdot}$ (100), 223 (41), 131 (10), 104 (91), 78 (34), 77 (28); HRCIMS *m/z* 283.0972 (calcd for $C_{17}H_{15}O_4$, 283.0970); CD (EtOH) λ_{ext} ($\Delta\epsilon$) 207 (+11.5), 225 (−3.3), 240 (+2.3), 260 (−0.3), 313 (+7.8), 355 (−5.1).

3.2.5. Cryptocaryanone B 6. Amorphous yellow powder, $[\alpha]_D^{25}=+288.6°$ (*c* 0.50, $CHCl_3$); UV (EtOH) λ_{max} (ϵ) 323

Table 7. Crystal data and structure refinement for bromocryptocaryone **3** and bicaryanone D **11**

Compound	Bromocryptocaryone **3**	Bicaryanone D **11**
Formula	$C_{17}H_{13}BrO_4$	$C_{34}H_{30}O_9$
Fw	361.18	582.58
Temperature (K)	293	293
Crystal system	Orthorhombic	Orthorhombic
Space group	*P* 212121	*P* 212121
a (Å)	7.0016(3)	6.8745(2)
b (Å)	13.0781(11)	18.4001(8)
c (Å)	16.6832(14)	22.2698(9)
α (°)	90	90
β (°)	90	90
γ (°)	90	90
V (Å^3)	1527.6(2)	2816.9(2)
Z	4	4
d_x (Mg/m^3)	1.570	1.374
μ (mm^{-1})	2.706	0.100
F(000)	728	1224
Crystal size (mm)	0.05×0.25×0.40	0.25×0.30×0.35
Range (°)	2.90–31.38	1.43–27.48
Index ranges	$-6\leq h\leq 7$	$-8\leq h\leq 8$
	$-19\leq k\leq 19$	$-23\leq k\leq 23$
	$-24\leq l\leq 24$	$-28\leq l\leq 28$
$N_{collect}$	4153	6377
N_{indt}	4153[*R*(int)=0.0000]	6377[*R*(int)=0.0000]
N_{obs}	2742[$I>2\sigma$ (*I*)]	3584[$I>2\sigma$ (*I*)]
R1 [$I>2\sigma$ (*I*)]	0.0482	0.0632
*wR*2	0.0798	0.1558

(6081), 201 (20155) nm; IR ($CHCl_3$) ν_{max} 1783, 1662, 1591, 1429, 1371, 1300, 11168, 894 cm^{-1}; ^{1}H NMR ($CDCl_3$, 250 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 75 MHz) see Table 2; EIMS *m/z* 282 [M]$^{+\cdot}$ (100), 223 (38), 131 (12), 104 (95), 78 (27), 77 (23); HRCIMS *m/z* 283.0954 (calcd for $C_{17}H_{15}O_4$, 283.0970); CD (EtOH) λ_{ext} ($\Delta\epsilon$) 205 (+3.7), 246 (+4.3), 274 (+5.1), 326 (+3.6).

3.2.6. Bicaryanone A 8. Amorphous white powder, $[\alpha]_D^{25}$=+269.4° (*c* 0.83, $CHCl_3$); UV (EtOH) λ_{max} (ϵ) 272 (10743), 202 (35793) nm; IR ($CHCl_3$) ν_{max} 1785, 1721, 1671, 1652, 1616, 1402, 1361, 1300, 1168, 1052, 906 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) see Table 3; ^{13}C NMR ($CDCl_3$, 75 MHz) see Table 4; EIMS *m/z* 564 [M]$^{+\cdot}$ (24), 282 (69), 223 (51), 131 (53), 105 (26), 104 (100), 103 (55), 91 (21), 78 (37), 77 (37); HRCIMS *m/z* 565.1847 (calcd for $C_{34}H_{29}O_8$, 565.1862); CD (EtOH) λ_{ext} ($\Delta\epsilon$) 200 (+60.6), 270 (+37.9), 306 (−12.7).

3.2.7. Bicaryanone B 9. Amorphous white powder, $[\alpha]_D^{25}$=+154.3° (*c* 0.70, $CHCl_3$); UV (EtOH) λ_{max} (ϵ) 270 (11909), 201 (40129) nm; IR ($CHCl_3$) ν_{max} 1784, 1724, 1672, 1649, 1617, 1399, 1362, 1301, 1167, 1057, 1003, 910 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) see Table 3; ^{13}C NMR ($CDCl_3$, 75 MHz) see Table 4; EIMS *m/z* 564 [M]$^{+\cdot}$ (75), 520 (14), 480 (8), 460 (6), 452 (8), 376 (10), 282 (100), 281 (37), 223 (92), 131 (96), 105 (47), 104 (96), 103 (97), 91 (39), 78 (58), 77 (57); HRCIMS *m/z* 565.1868 (calcd for $C_{34}H_{29}O_8$, 565.1862); CD (EtOH) λ_{ext} ($\Delta\epsilon$) 269 (+37.0), 302 (−3.7), 340 (+0.8).

3.2.8. Bicaryanone C 10. Amorphous white powder, $[\alpha]_D^{25}$=+287.8° (*c* 0.93, $CHCl_3$); UV (EtOH) λ_{max} (ϵ) 274 (9139), 201 (32991) nm; IR ($CHCl_3$) ν_{max} 1785, 1721, 1674, 1652, 1618, 1399, 1362, 1300, 1228, 1169, 1052, 906 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) see Table 3; ^{13}C NMR ($CDCl_3$, 75 MHz) see Table 4; EIMS *m/z* 564 [M]$^{+\cdot}$ (37), 522 (7), 520 (8), 460 (6), 282 (29), 281 (37), 223 (28), 131 (92), 104 (100), 103 (53), 91 (22), 78 (21), 77 (24); HRCIMS *m/z* 565.1859 (calcd for $C_{34}H_{29}O_8$, 565.1862); CD (EtOH) λ_{ext} ($\Delta\epsilon$) 223 (−3.9), 273 (+19.8), 310 (−5.6).

3.2.9. Bicaryanone D 11. Unstable white crystals from ethylacetate (Fig. 2, Table 7), $[\alpha]_D^{25}$=+162.2° (*c* 0.93, $CHCl_3$); UV (CH_3CN) λ_{max} (ϵ) 269 (10784), 208 (23603) nm; IR ($CHCl_3$) ν_{max} 1782, 1722, 1677, 1647, 1618, 1410, 1370, 1301, 1227, 1182, 1055, 1003, 870 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) see Table 3; ^{13}C NMR ($CDCl_3$, 75 MHz) see Table 4; EIMS *m/z* 564 [M]$^{+\cdot}$ (32), 522 (6), 520 (5), 460 (4), 282 (20), 281 (16), 223 (18), 131 (64), 104 (100), 103 (40), 91 (24), 78 (25), 77 (30); HRCIMS *m/z* 565.1878 (calcd for $C_{34}H_{29}O_8$, 565.1862); CD (EtOH) λ_{ext} ($\Delta\epsilon$) 222 (−13.3), 271 (+17.3), 337 (−1.2).

3.2.10. Chalcocaryanone A 12. Amorphous yellow powder, $[\alpha]_D^{25}$=+123.1° (*c* 0.32, $CHCl_3$); UV (EtOH) λ_{max} (ϵ) 378 (10522), 297 (9576), 267 (11394), 200 (34397) nm; IR ($CHCl_3$) ν_{max} 3026, 1782, 1719, 1643, 1627, 1578, 1415, 1371, 1301, 1224, 1209, 1056 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) see Table 5; ^{13}C NMR ($CDCl_3$, 75 MHz) see Table 6; EIMS *m/z* 564 [M]$^{+\cdot}$ (15), 460 (5), 282 (19), 238 (11), 223 (17), 160 (11), 150 (20), 131 (28), 104 (100), 103 (68), 91 (25), 78 (43), 77 (57); HRCIMS *m/z* 565.1858 (calcd for $C_{34}H_{29}O_8$, 565.1862); CD (EtOH) λ_{ext} ($\Delta\epsilon$) 228 (−10.8), 225 (+2.7), 246 (+6.1), 297 (+5.8).

3.2.11. Chalcocaryanone B 13. Amorphous yellow powder, $[\alpha]_D^{25}$=+41.2° (*c* 1.01, $CHCl_3$); UV (EtOH) λ_{max} (ϵ) 376 (16789), 268 (13170), 200 (45491) nm; IR ($CHCl_3$) ν_{max} 3019, 1782, 1719, 1673, 1648, 1628, 1578, 1415, 1370, 1301, 1224, 1180, 1053 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) see Table 5; ^{13}C NMR ($CDCl_3$, 75 MHz) see Table 6; EIMS *m/z* 564 [M]$^{+\cdot}$ (21), 460 (10), 282 (42), 238 (29), 223 (25), 160 (21), 150 (42), 131 (100), 104 (88), 103 (54), 91 (25), 78 (38), 77 (46); HRCIMS *m/z* 565.1878 (calcd for $C_{34}H_{29}O_8$, 565.1862); CD (EtOH) λ_{ext} ($\Delta\epsilon$) 202 (+53.6), 232 (−4.7), 247 (+14.7), 332 (−3.1).

3.2.12. Chalcocaryanone C 14. Amorphous white powder, $[\alpha]_D^{25}$=+139.5° (*c* 0.60, $CHCl_3$); UV (EtOH) λ_{max} (ϵ) 304 (158000), 201 (25930) nm; IR ($CHCl_3$) ν_{max} 1788, 1730, 1672, 1609, 1596, 1576, 1398, 1333, 1304, 1208, 1171, 1050, 980 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) see Table 5; ^{13}C NMR ($CDCl_3$, 75 MHz) see Table 6; EIMS *m/z* 564 [M]$^{+\cdot}$ (36), 460 (14), 432 (6), 131 (100), 104 (30), 103 (42), 91 (14), 78 (10), 77 (16); HRCIMS *m/z* 565.1892 (calcd for $C_{34}H_{29}O_8$, 565.1862); CD (EtOH) λ_{ext} ($\Delta\epsilon$) 271 (+19.7), 303 (−1.1), 324 (+2.0).

3.2.13. Chalcocaryanone D 15. Amorphous white powder, $[\alpha]_D^{25}$=+160.7° (*c* 1.06, $CHCl_3$); UV (EtOH) λ_{max} (ϵ) 303 (21098), 208 (17473) nm; IR ($CHCl_3$) ν_{max} 1789, 1730, 1673, 1617, 1595, 1576, 1392, 1332, 1303, 1228, 1172, 1049, 978 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) see Table 5; ^{13}C NMR ($CDCl_3$, 75 MHz) see Table 6; EIMS *m/z* 564 [M]$^{+\cdot}$ (47), 460 (12), 432 (6), 208 (25), 131 (100), 104 (35), 103 (43), 91 (20), 77 (23); HRCIMS *m/z* 565.1871 (calcd for $C_{34}H_{29}O_8$, 565.1862); CD (EtOH) λ_{ext} ($\Delta\epsilon$) 220 (+18.4), 227 (−15.2), 277 (+20.6), 306 (+16.9), 343 (−3.4).

3.2.14. Chemical correlation of 5 and 6 with 1. A solution of cryptocaryone **1** (53 mg) in a mixture of EtOH (2 mL), acetic acid (2 mL) and H_2O (2 mL) was refluxed for 20 h. The reaction mixture was treated with an aqueous solution of $NaHCO_3$ and extracted with CH_2Cl_2. Purification of the extract by preparative TLC plates yielded racemic **7** (34 mg) and a mixture of dihydroflavanones (10 mg) identical to natural **5** and **6**.

Compound **7**: amorphous white powder, $[\alpha]_D^{25}=0°$ (*c* 0.60, $CHCl_3$); UV (EtOH) λ_{max} (ϵ) 325 (2427), 259 (4824), 201 (30778) nm; IR ($CHCl_3$) ν_{max} 3520, 1715, 1683, 1603, 1578, 1474, 1443, 1409, 1372, 1319, 1293, 1132, 1094, 899 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) 7.48 (5H, m, Ar-H), 7.45 (1H, m, H-7), 7.06 (1H, d, *J*=8.3 Hz, H-6), 6.90 (1H, d, *J*=7.4 Hz, H-8), 5.50 (1H, dd, *J*=13.4, 2.8 Hz, H-2), 4.06 (1H, m, H-11), 3.15 (1H, dd, *J*=16.6, 13.4 Hz, H-3a), 2.90 (1H, dd, *J*=16.6, 13.4 Hz, H-3b); ^{13}C NMR ($CDCl_3$, 75 MHz) 193.9 (C-4), 173.7 (C-12), 163.0 (C-9), 138.7 (C-1′), 136.9 (C-5), 135.4 (C-7), 128. 9 (C-3′, C-4′ and C-5′), 126.2 (C-6′), 126.17 (C-2′), 79.0 (C-2), 45.6 (C-3); ESIMS *m/z* 587 [2M+Na^+], 305 [M+Na^+].

3.3. Crystallographic data collection and refinement of the structures

A needle-shaped bright-orange crystal of bromocryptocaryone **3** with dimensions 0.05×0.25×0.40 mm^3 and a block-shaped colourless crystal of bicaryanone D **11** with dimensions 0.25×0.30×0.35 mm^3 were chosen for X-ray diffraction experiments. Diffraction data were collected on a ENRAF NONIUS CCD-based diffractometer at room temperature using graphite monochromated MoKα radiation (λ=0.71073). The reflections covered a full sphere of reciprocal space, the crystal-to-detector distance was 40 mm. The complete data-collection strategy and crystallographic details are summarized in Table 7. Cell parameters were retrieved using Kappa CCD software. Both complexes crystallized in the orthorhombic space group *P* 212121. Data reduction was performed using the same software. Both structures were solved by direct methods using the SHELX86[6] program and refined with SHELX93.[7] The drawings were prepared with ORTEP II.[8] For compound **3**, hydrogen atom positions were found in differences Fourier maps and were isotropically refined. Final weighting scheme was $w = 1/[\sigma^2(F_0^2) + (0.0237P)^2 + 0.9510P]$, where $P = (F_0^2 + 2F_c^2)/3$. The final refinement of this model was continued until convergence when $R1 = 0.0482$ for $F^2>2(F^2)$ and $R_w = 0.0798$. The final difference map showed the largest residual peaks of 0.371 and -0.394 e$Å^{-3}$. Compound **11** is realised as a crystallohydrate. The structure (and the non-hydrogen atoms of water) was refined anisotropically by full-matrix least-squares approximation based on F^2 (in *P* 212121 space group). Hydrogen atom positions were calculated by assuming geometrical positions were included in the structural model. Final weighting scheme was $w = 1/[\sigma^2(F_0^2) + (0.1386P)^2 + 0.7844P]$, where $P = (F_0^2 + 2F_c^2)/3$. The final refinement of this model was continued until convergence when $R1 = 0.0632$ for $F^2>2(F^2)$ and $R_w = 0.1558$. The final difference map showed the largest residual peaks of 0.448 and -0.294 e$Å^{-3}$. The observed high-temperature factors of H_2O indicate a low accuracy in the determination of the position of the *O* atom. This might arise from partial disorder of H_2O, but refinement of a model based on disorder was unsuccessful. Consequently, we attributed the high-temperature factors to the strong thermal vibrations of the H_2O. The crystallographic data have been deposited at the Cambridge Crystallographic Data Centre and allocated the deposition numbers 157760 and 157761.

3.4. Cytotoxicity assay

KB cells, coming from a mouth epidermoid carcinoma, were originally obtained from the American type culture collection.[9] The assays were performed according to a published technique.[10] The human erythroleukemia cells K562-S and K562/DOX (K562-R) were kindly provided by Dr Tapiero (Faculté de Pharmacie, Chatenay-Malabry, France). The original K562 cell line consists of Philadelphia chromosome-positive cells obtained from a patient with chronic myelogenous leukemia.[11] The K562-R cell line shown to overexpress *P*-glycoprotein,[12] is derived from the K562-S through serial passages in the presence of doxorubucin. Both cell lines were grown as suspensions in RPMI-1640 medium containing 10% fetal calf serum, 2 mM L-glutamine, 60 μg/ml penicillin G and streptomycin sulfate and 40 μg/ml gentamycin. For the assays, 25,000 cells under a volume of 1 ml of medium were seeded in each well of 24-well Nunc microplates and various concentrations of the tested compound were added immediately to the wells under a volume of 0.1 ml. Cultures were incubated for 3 days at 37°C in a 5% CO_2–95% air incubator, and cell viability was determined by the MTT colorimetric assay.[13]

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We dedicate this work to Dr Mai Van Tri, who initiated with one of us (T. S.) the cooperation for the chemical study of Vietnamese flora, and died unexpectedly in January 1999.

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